

## Examining the potential for porcine-derived islet cells to harbour viral pathogens

Crossan, Claire; O'Hara, Zoe; Mourad, Nizar; Gianello, Pierre ; Scobie, Linda

*Published in:*  
Xenotransplantation

*DOI:*  
[10.1111/xen.12375](https://doi.org/10.1111/xen.12375)

*Publication date:*  
2018

*Document Version*  
Author accepted manuscript

[Link to publication in ResearchOnline](#)

### *Citation for published version (Harvard):*

Crossan, C, O'Hara, Z, Mourad, N, Gianello, P & Scobie, L 2018, 'Examining the potential for porcine-derived islet cells to harbour viral pathogens', *Xenotransplantation*, vol. 25, no. 2, e12375.  
<https://doi.org/10.1111/xen.12375>

### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

### **Take down policy**

If you believe that this document breaches copyright please view our takedown policy at <https://edshare.gcu.ac.uk/id/eprint/5179> for details of how to contact us.

**Title:**

**Examining the potential for porcine derived islet cells to harbour viral pathogens**

**Authors:**

Claire Crossan<sup>1</sup>, Zoe O'Hara<sup>1</sup>, Nizar Mourad<sup>2</sup>, Pierre Gianello<sup>2</sup>, Linda Scobie<sup>1</sup>

1- School of Health and Life Sciences, Glasgow Caledonian University, Glasgow, G4 0BA, UK

2- Pôle de chirurgie expérimentale et transplantation, Université catholique de Louvain,  
1200 Brussels, Belgium

**Abstract:**

With an onus on safety in the potential use of porcine islet cells as a treatment for diabetes, the use of animals lacking exogenous pathogens is clearly important and multi-level screening strategies have been presented on testing animals and the product.

In this study, we wished to investigate whether islet cells indeed harboured the same viral pathogens of concern in the source animal. PMBC and islet cells from both adult and neonatal source animals were directly compared and tested for PCMV, PLHV, PCV2, PPV and HEV using both molecular and serological assays.

Adult PBMC were found positive for all viruses with the exception of PCV2 and HEV. Neonatal PBMC were only found positive for PCMV and HEV. All animals were found negative for HEV antibodies.

Interestingly, islet cells were negative for all viruses tested regardless of status in the animal derived PBMC. Given that other laboratories have demonstrated the lack of virus detection during culture of islets, this study also demonstrates that the hygiene status of the herd may not reflect the status of the product. This is important for establishing guidelines for any risk evaluation and mitigation process utilised during product manufacture.

## 24    **Introduction:**

25    The risk of zoonotic disease transmission to human recipients during xenotransplantation is  
26    considered one of the barriers to xenotransplantation's transition to clinical practice<sup>1</sup>. Such risks can  
27    be difficult to eliminate, particularly in the case of Porcine Endogenous Retrovirus. However this risk  
28    may be reduced by the selection of low PERV expressing donor pigs or eliminated by the creation of  
29    PERV free pigs via genetic manipulation<sup>2</sup>. The risk posed by exogenous pathogens can be minimised  
30    by thorough screening of donor animals from barrier reared herds<sup>3</sup>. However, it is clear that certain  
31    pathogens can be difficult to detect, particularly for viruses that form latent infections, are highly  
32    divergent or for which sensitive and specific assays are not available<sup>4,5</sup>.

33    The encapsulation of porcine islets has been evaluated as a therapeutic modality for the treatment  
34    of diabetes and, despite the lack of evidence to date of the transmission of specific exogenous  
35    viruses to both animal models and humans, evaluation of the microbiological safety of porcine islets  
36    is still required. Indeed current methods have been evaluated<sup>6</sup>.

37    It is clear that different cells, tissues and organs vary in their potential to harbour infectious  
38    pathogens and this variation may play an important factor in assessing the risk of zoonotic infection  
39    in a particular xenotransplantation trial<sup>3,6</sup>. A number of reports have characterised the pathogen  
40    status of pigs<sup>7,8</sup>, however, whether an animal's pathogen profile obtained through blood screening is  
41    reflective of the pathogen profile of its tissues or organs has not been previously investigated. It is  
42    important to note that pathogens detected in the blood may not be present in the transplanted  
43    tissue and vice versa. Given the progression of porcine islet xenotransplantation towards clinical  
44    application, we felt it prudent to examine the potential for porcine islet cells to harbour exogenous  
45    pathogens in comparison to peripheral blood mononuclear cell (PBMC) samples from the same  
46    animal for several key viruses considered to have zoonotic potential<sup>9</sup>; Porcine Cytomegalovirus  
47    (PCMV), Porcine Parvovirus (PPV), Hepatitis E Virus (HEV), Porcine Lymphotropic Herpesvirus 1-3  
48    (PLHV1-3) and Porcine Circovirus 2 (PCV-2).

49

## 50 **Materials and Methods:**

51 Pigs (Belgian landrace) were provided by Rattlerow-Seghers Genetics (Ooigem, Belgium). Piglets  
52 were directly delivered to the islet isolation facility in Brussels. Adult pigs were housed in the A. de  
53 Marbaix center (Louvain-la-Neuve, Belgium). All experiments were conducted in accordance with the  
54 local ethical committee and carried out in accordance to EU Directive 2010/63/EU for animal  
55 experiments. Two age category of animal were tested; neonatal (14-21days) and adult (1-2 years,  
56 100-300kg). Neonatal animals had not yet undergone weaning from the sow. Blood samples were  
57 taken to prepare PBMCs. Briefly, 50 mL heparinized blood were centrifuged at room temperature  
58 over Histopaque 1077 from Sigma (Darmstadt, Germany). The opaque interface containing PBMCs  
59 was then aspirated, washed in PBS, pelleted and stored at -70°C. Pancreatic islet isolation was  
60 carried out as detailed elsewhere<sup>10</sup> using a method based on that described by Korbitt et al<sup>11</sup>.  
61 Collagenase V from Sigma was used for piglet pancreas digestion and collagenase NB8 from Serva  
62 (Heidelberg, Germany) was used for adult pancreas. We obtained 2087±151 islet equivalents (IEQ)/g  
63 pancreas (purity >75%) from neonatal piglets and 234±16 IEQ/g pancreas (purity >90%) from adult  
64 pigs (mean±SEM). Islet samples were taken after overnight culture in the case of adult islets and  
65 after 8 days in the case of neonatal islets. Corresponding serum samples were also collected from  
66 each animal and analysed using the swine HEV IgG ELISA (Wanti Beijing, Beijing, People's Republic of  
67 China). DNA was isolated from the PBMC and islet samples using the DNeasy mini kit (Qiagen,  
68 Crawley, UK). RNA was isolated from islet samples using the RNeasy mini kit (Qiagen, Crawley, UK).  
69 Viral RNA was isolated from serum using the Viral RNA mini kit (Qiagen, Crawley, UK). PCR assays  
70 were performed as previously described; PCMV<sup>12</sup>, PLHV1<sup>13</sup>, PLHV2<sup>14</sup>, PLHV3<sup>15</sup>, PCV2<sup>16</sup>, PPV<sup>17</sup> and  
71 HEV<sup>18</sup>, the sensitivity of these assays was determined to be 2copies, 10 copies, 2 copies, 10copies,  
72 5copies, 5 copies and 2.5IU per reaction, respectively. For DNA virus detection, each reaction  
73 contained 250ng of DNA. All samples were assessed for integrity and RNA was validated in serum  
74 and confirmed without contamination as described previously<sup>19</sup>. All animals had received a

75 vaccination against mycoplasma (Stellamune, Elanco) at 14 days of age. Adult pigs had received a  
76 vaccination against; porcine parvovirus and Erysipelothrix rhusiopathiae (Porcilis ERY-PARVO  
77 (Intervet International)) at 6 months of age and again after each farrowing.

78

## Results and discussion:

As indicated, adult and neonatal animals were screened for PCMV, PLHV, PCV, PPV and HEV utilising corresponding PBMC, serum and islet samples. Overall, none of the viruses were found in the islet samples despite their presence in the PBMC or serum samples analysed. PCV-2 was not found in any of the animals tested. PCMV was only detected in the PBMC and the remaining data is summarised in Table 1.

As shown in Table 1, no islet sample tested positive for any herpes virus, despite several animals testing positive in the PBMC for herpes viruses, ranging from 2.9% for PLHV2 to 38.1% for PLHV3. In humans, cases of acute pancreatitis associated with herpes viruses such as CMV<sup>20</sup>, Varicella-Zoster virus (VZV)<sup>21</sup> and herpes simplex virus (HSV)<sup>22</sup> do occur but are rare, although the cell type affected within the pancreas is not known. However, several reports have examined CMV in immunosuppressed CMV negative patients receiving islet allografts from CMV positive donors and found a complete absence of CMV transmission in all recipients (n=12 and n=4)<sup>23</sup> supporting our data that islet cells do not commonly harbour herpes viruses.

Hepatitis E has also been associated with acute pancreatitis in humans<sup>24</sup>, but as no islet sample tested positive for HEV, and only 1 serum sample tested positive for HEV, it is not possible to draw any conclusions regarding the potential for islet cells to harbour HEV. In addition, all animals were negative for antibodies to HEV.

No neonatal animals tested positive for PPV and only one adult pig tested positive for PPV, although adult animals were vaccinated for PPV. There are several possible explanations for this; possibly this pig was a vaccine non-responder, its immunity had waned since vaccination, the animal had recently been vaccinated and the PCR detected the attenuated vaccine strain or the vaccine the animal received did not protect against the strain the animal was infected with. All animals tested PCV2 negative, suggesting that the virus was not circulating in these herds.

103

104 There was a higher prevalence of herpes viruses in the adult population in comparison to the  
105 neonatal population. This is concurrent with increased risk of exposure to viruses with increasing  
106 age. This data also supports the suggestion of early weaning as a measure to reduce the risk of  
107 herpes transmission to neonates. The duration of islet culture following isolation varied between  
108 however, as no islet sample tested positive for any virus, it is not possible to determine if the period  
109 of culture after isolation could impact on the detection of contaminating viruses.

110 In conclusion, this data suggests that porcine islet cells do not harbour porcine herpes viruses; PCMV  
111 and PLHV1-3. Although further confirmation of this finding via in-vitro culture studies, to examine  
112 the permissibility of porcine islet cells to herpesvirus infection, may prove useful. However, despite  
113 having used the most sensitive assays available to us, we cannot exclude the possibility of low level  
114 infection of islet cells by herpes viruses, below the level of detection of our assays.

115 This study indicates that the risk of porcine islet pathogen contamination is low, regardless of age of  
116 harvest, and that, irrespective of the multi-level testing strategy, testing of the end product will be  
117 an important requirement. This report has identified an absence of correlation between the  
118 pathogen profiles of porcine PBMCs and islets, indicating that product screening in  
119 xenotransplantation, as opposed to ante mortem donor screening, provides a safer and more  
120 reliable approach. Updates to current regulations have been suggested<sup>6,25</sup> and information on the  
121 relevance of viral pathogens in specific xenotransplant products will aid to advise this.

122

## References:

1. First WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials: Changsha, China, 19-21 November 2008. The Changsha Communiqué. *Xenotransplantation*. 16(2):61-63. doi:10.1111/j.1399-3089.2009.00520.x.
2. Niu D, Wei H-J, Lin L, et al. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. *Science (80- )*. 2017;357(6357):1303-1307. doi:10.1126/science.aan4187.
3. Spizzo T, Denner J, Gazda L, et al. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes-Chapter 2a: source pigs-preventing xenozoonoses. *Xenotransplantation*. 2016;23(1):25-31. doi:10.1111/xen.12223.
4. Lemmon GH, Gardner SN. Predicting the sensitivity and specificity of published real-time PCR assays. *Ann Clin Microbiol Antimicrob*. 2008;7(1):18. doi:10.1186/1476-0711-7-18.
5. Denner J. Sensitive methods and improved screening strategies are needed for the detection of pig viruses. *Xenotransplantation*. 2017;24(3). doi:10.1111/xen.12303.
6. Schuurman H-J. Microbiological safety of clinical xenotransplantation products: monitoring strategies and regulatory aspects. A commentary. *Xenotransplantation*. 2016;23(6):440-443. doi:10.1111/xen.12280.
7. Morozov VA, Plotzki E, Rotem A, Barkai U, Denner J. Extended microbiological characterization of Göttingen minipigs: porcine cytomegalovirus and other viruses. *Xenotransplantation*. 2016;23(6):490-496. doi:10.1111/xen.12265.
8. Morozov VA, Heinrichs G, Denner J. Effective Detection of Porcine Cytomegalovirus Using Non-Invasively Taken Samples from Piglets. *Viruses*. 2017;9(1). doi:10.3390/v9010009.
9. Crossan C, Takeuchi Y, Scobie L. Possible Viral Zoonoses in Xenotransplantation. *eLS*. 2014.



doi:10.1002/9780470015902.a0020708.pub2.

10. Mourad N, Perota A, Xhema D, Galli C, Gianello P. Transgenic expression of glucagon-like peptide 1 (GLP-1) and activated muscarinic receptor (M3R) significantly improves pig islet secretory function. *Cell Transplant*. November 2016. doi:10.3727/096368916X693798.
11. Korbitt GS, Elliott JF, Ao Z, Smith DK, Warnock GL, Rajotte R V. Large scale isolation, growth, and function of porcine neonatal islet cells. *J Clin Invest*. 1996;97(9):2119-2129. doi:10.1172/JCI118649.
12. Hamel AL, Lin L, Sachvie C, Grudeski E, Nayar GP. PCR assay for detecting porcine cytomegalovirus. *J Clin Microbiol*. 1999;37(11):3767-3768. <http://www.ncbi.nlm.nih.gov/pubmed/10523598>.
13. Ehlers B, Ulrich S, Goltz M. Detection of two novel porcine herpesviruses with high similarity to gammaherpesviruses. *J Gen Virol*. 1999;80 ( Pt 4):971-978. doi:10.1099/0022-1317-80-4-971.
14. Ulrich S, Ehlers B, Goltz M. Detection of two novel porcine herpesviruses with high similarity to gammaherpesviruses. *J Gen Virol*. 1999;80(4):971-978. doi:10.1099/0022-1317-80-4-971.
15. Chmielewicz B, Goltz M, Franz T, et al. A novel porcine gammaherpesvirus. *Virology*. 2003;308(2):317-329. doi:10.1016/S0042-6822(03)00006-0.
16. Puvanendiran S, Stone S, Yu W, et al. Absence of porcine circovirus type 1 (PCV1) and high prevalence of PCV 2 exposure and infection in swine finisher herds. *Virus Res*. 2011;157(1):92-98. doi:10.1016/j.virusres.2011.02.012.
17. Soares RM, Durigon EL, Bersano JG, Richtzenhain LJ. Detection of porcine parvovirus DNA by the polymerase chain reaction assay using primers to the highly conserved nonstructural protein gene, NS-1. *J Virol Methods*. 1999;78(1-2):191-198.

169 <http://www.ncbi.nlm.nih.gov/pubmed/10204709>.

170 18. Erker JC, Desai SM, Mushahwar IK. Rapid detection of Hepatitis E virus RNA by reverse  
 171 transcription-polymerase chain reaction using universal oligonucleotide primers. *J Virol*  
 172 *Methods*. 1999;81:109-113.

173 19. Mourad NI, Crossan C, Cruikshank V, Scobie L, Gianello P. Characterization of porcine  
 174 endogenous retrovirus expression in neonatal and adult pig pancreatic islets.  
 175 *Xenotransplantation*. 2017;epub ahead:e12311. doi:10.1111/xen.12311.

176 20. Chan A, Bazerbachi F, Hanson B, Alraies MC, Duran-Nelson A. Cytomegalovirus hepatitis and  
 177 pancreatitis in the immunocompetent. *Ochsner J*. 2014;14(2):295-299.  
 178 <http://www.ncbi.nlm.nih.gov/pubmed/24940147>.

179 21. Kulasegaran S, Wilson EJ, Vasquez L, Hulme-Moir M. Varicella zoster virus: a rare cause of  
 180 acute pancreatitis in an immunocompetent child. *BMJ Case Rep*. 2016;2016. doi:10.1136/bcr-  
 181 2015-213581.

182 22. Konstantinou GN, Liatsos CN, Patelaros EG, Karagiannis SS, Karnesis LI, Mavrogiannis CC.  
 183 Acute pancreatitis associated with herpes simplex virus infection: report of a case and review  
 184 of the literature. *Eur J Gastroenterol Hepatol*. 2009;21(1):114-116.  
 185 doi:10.1097/MEG.0b013e3283005890.

186 23. Hafiz MM, Poggioli R, Caulfield A, et al. Cytomegalovirus Prevalence and Transmission After  
 187 Islet Allograft Transplant in Patients with Type 1 Diabetes Mellitus. *Am J Transplant*.  
 188 2004;4(10):1697-1702. doi:10.1111/j.1600-6143.2004.00557.x.

189 24. Bazerbachi F, Haffar S, Garg SK, Lake JR. Extra-hepatic manifestations associated with  
 190 hepatitis E virus infection: a comprehensive review of the literature. *Gastroenterol Rep*.  
 191 September 2015;gov042. doi:10.1093/gastro/gov042.

192 25. Cooper DKC, Pierson RN, Hering BJ, et al. Regulation of Clinical Xenotransplantation—Time  
193 for a Reappraisal. *Transplantation*. 2017;101(8):1766-1769.  
194 doi:10.1097/TP.0000000000001683.

195

196 This work was supported by a European Commission FP7 funded project XENOSLET (*project*  
197 *number 601827*).

198